Chapter Five

Bioreactor Design

INTRODUCTION

- Desigining of bioreactors: for production of vaccines, proteins, organics acids, amino acids and antibiotics; enzymatic or microbial bio-transformations; bioremediation, etc.
- A production facility usually has a train of bioreactors ranging from 20 L to 250,000 L. The bioreactors are arranged in the series of increasing sizes, starting from small cultures to the final production culture.

BIOREACTOR DESIGN CONFIGURATIONS

Stirred Tank Reactors

Features:

- Microbial reactors generally have 4 baffles from the walls to prevent vortexing of the fluid, the baffle width is 1/10 or 1/12 of the tank diameter.
- The vortex and circular flow result in little mixing between fluids at different heights. At high speeds the vortex may reach down to the impeller so that gas from the surrounding is drawn into the liquid→high

mechanical stress in the stirrer shaft, bearings and seal.

- Bioreactors for animal cell cultures usually do not have baffles (especially for small scale reactors) to reduce turbulence.
- The aspect ratio (height-to-diameter ratio) of the vessel is 3-5 for microbial cultures but is normally less than 2 for animal cell culture.
- Sparger: gas is sparged at the bottom using a perforated pipe ring sparger.

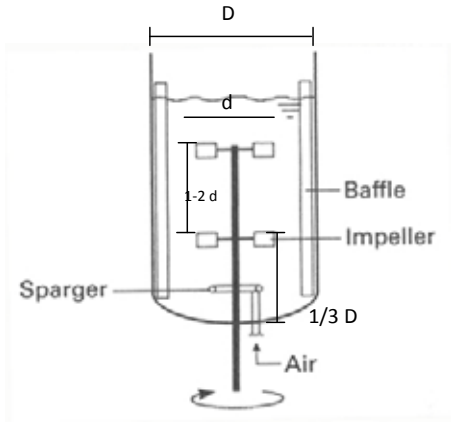

Fig. 90: Showing the design of a STR

- Number of impellers depends on the aspect ratio. The bottom impeller is located at a distance about 1/3 of the tank diameter above the bottom of the tank. Additional impellers are spaced approximately 1 to 2 impeller diameter (d) distances apart.
- The superficial aeration velocity (the volume flow rates of gas divided by the cross-sectional area of the vessel) in stirred vessel must be lower than that can flood the impeller (an impeller is flooded when it receives more gas than it can effectively disperse) otherwise the mixing is poor. Superficial aeration velocities generally do not exceed 0.05 m/s.

Bioreactor Systems

Since bioreactors are used for commercial purposes, process monitoring and control play an important role. The idea behind control is simple: perform a measurement of a process parameter, compare it with the set point (*i.e.* optimal desired value) and generate an actuator output to influence this parameter.

In bioreactor vessels used for classical applications (*e.g.* fermentation), stirring is the most important process parameter, because cells need to be suspended homogeneously in the reactor and sufficient oxygen must be dissolved into the media. Secondary to this it is important to achieve control of the oxygen concentration to meet the increasing oxygen consumption during cultivation. Thirdly, pH should be maintained at a constant level to reduce the

effect of toxic by-products (*e.g.* organic acids or ammonia). Going to larger and more complicated systems, other parameters will follow. All in all, it is almost impossible to cultivate micro-organisms or animal cells in bioreactors without control. The benefits of measurement and control are evident:

- Reduced labour (in general)
- Optimal process conditions (higher quality and uniformity, better reproducibility)
- Higher efficiency (optimal usage of media and higher cell concentration)
- Better quality control and monitoring (helps GMP documentation)
- Reduced risk of human errors and contamination (GMP risk analysis

The comparison with the set point and generation of output signal is performed using control intelligence (proportional integral derivative 'PID' control) in the form of a programmable logic controller (PLC) or a PC. These steps are repeated continuously during the cultivation process, in such a way that the process parameter always stays close to the set point. In practice, a control system can be more complicated, because parameters are coupled (*e.g.* CO_2 also affects pH and stirring will affect O_2 transfer and temperature) and more actuators can be used to reach a set point (*e.g.* cascade control for O_2 by controlling air, O_2 and stirrer speed). Different control strategies can be implemented in the controller software and the user can select these features through the user interface.

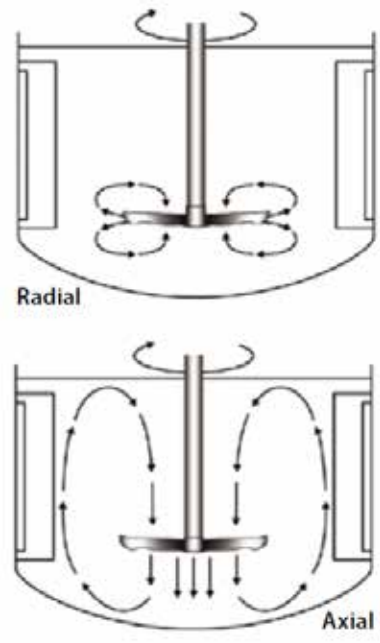

Fig 91: Showing the Axial and Radical flow models

Impellers

When growing microbes or animal cells in a stirred tank reactor, it is critical to choose the impeller type that is best suited to your process. Select the wrong impeller, and you could make chop suey of your filamentous fungi. Pick the right impeller, and you could greatly increase yields of your fussy mammalian cultures such as Chinese

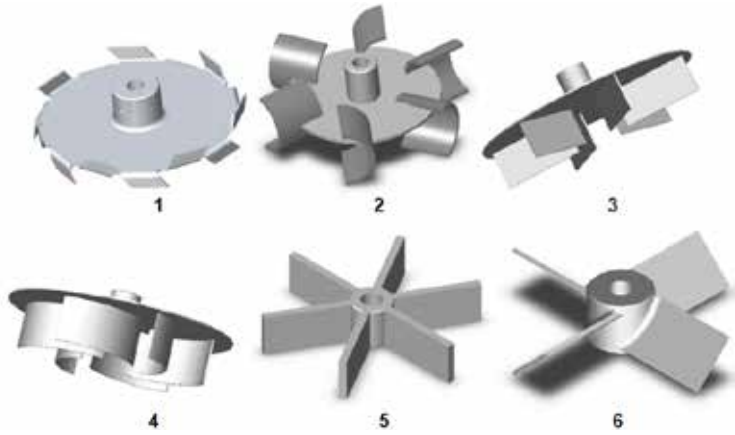

Fig. 92: The radial impellers used in experiments (1 - Disperser sawtooth, 2 - Smith turbine, 3 - Pumper mixer, 4 - Curved bladed turbine, 5 - Paddle with six blades, 6 - Pitched bladed turbine).

hamster ovary (CHO) and Vero kidney epithelial cells. With a wide range of impeller designs to choose from, how do you tell which is right for your application? Here we describe six commonly used fermentor and bioreactor impellers, explain how they work, and identify which may perform best for culturing certain animal, insect, plant, yeast, and bacterial cell lines.

How Blade Orientation Affects Mixing: All impellers are designed to homogeneously mix cells, gases, and nutrients throughout the culture vessel. The mixing action evenly distributes oxygen and nutrients to cells for healthy growth, keeps them from settling to the bottom of the vessel, and helps to maintain a uniform culture temperature. Depending on the impeller type you select, mixing will be imparted as a radial flow, axial flow, or a combination of the two. As Figure 91 shows, radial flow occurs when fluid is pushed away from the impeller's axis toward the vessel wall. Axial flow occurs when fluid is pushed up or down along the axis or shaft of the impeller. The orientation of an impeller (left- or right-handed) and its agitating direction determine whether the direction of axial flow is up or down. A right-handed impeller option will push fluid in an upward direction toward the top of the vessel if agitation is clockwise (as viewed from the top). A left-handed option paired with a clockwise agitation will push fluid down toward the bottom of the vessel. Therefore, when positioning blades on an impeller shaft, it's important to know which direction your impeller will be turning (clockwise or counterclockwise). To increase mixing action in some applications, one impeller blade may be oriented for up

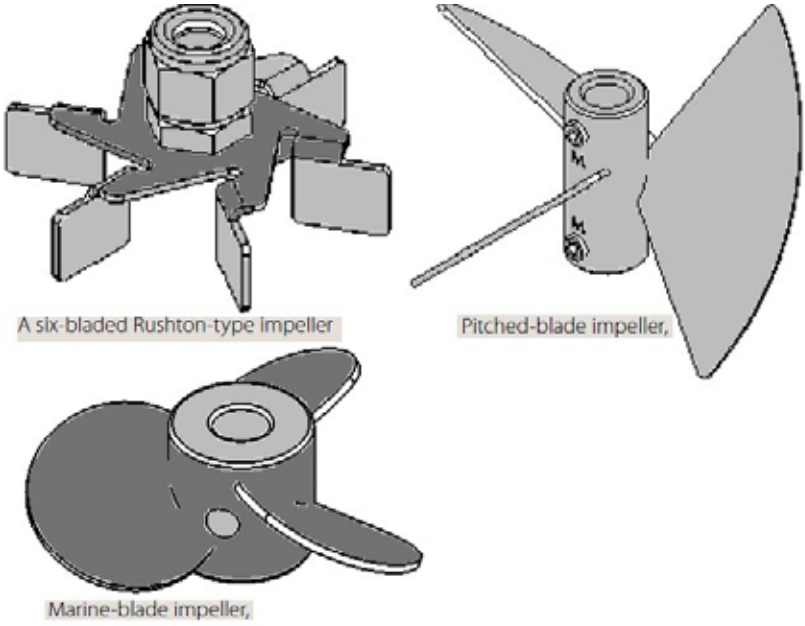

Fig. 92A: Some more Impellers.

flow while the other is positioned for down flow. Although there is no right or wrong way to position an impeller blade(s), reversing flow direction could improve yields in some instances.

Rushton Impellers for Fermentation

J. H. Rushton originally called the impellers he designed flat-bladed turbines. So Rushton impeller is today the most common generic term applied to flat-bladed or disk-turbine impellers. Their blades are flat and set vertically along an agitation shaft, which produces a unidirectional radial flow. Rushton and Rushton-type impellers are commonly used in fermentations of cell lines that are not considered shear sensitive, including yeasts, bacteria, and some fungi.

Pitched-Blade Impellers for Shear-Sensitive Cells

The blades on pitched-blade impellers (Figure 92A) are flat and set at ~45° angles, which produces a simultaneous axial and radial flow. This combination provides better overall mixing and creates a higher oxygen mass transfer rate ($K_L a$) than that of unidirectional marine blade impellers. Pitched-blade impellers are lows hear impellers designed to gently mix the contents of a

culture without causing cell damage. They are most often used with mammalian, insect, or other shear-sensitive cell lines growing in suspension or with the aid of micro-carriers. These impellers are often used in batch or fed-batch cultures, but they can also be used for continuous and perfusion processes. Because of their proficient mixing design, pitched-blade impellers are also widely used in fermentation processes that involve highly viscous cultures, such as filamentous bacteria and fungi, as well as in some anaerobic biofuels processes.

Gentle Marine-Blade Impellers

The leading face of the blades on a marine-blade impeller (Figure 92A) can be flat or concave, whereas their back sides are convex. This produces an axial flow. Like pitched-blade impellers, marine blade impellers are used for applications that require gentle mixing without causing cell damage. Due to the unidirectional flow, however, the $K_l a$ values of marine-blade impellers tend to be slightly lower than those of impellers that produce both axial and radial mixing.

Spin Filters

Spin filters are retention devices commonly used to keep cells inside a vessel during continuous or perfusion culture. In New Brunswick Scientific (NBS) bioreactors, for example, spin filters with low-shear marine blade impellers are designed for suspension and micro-carrier applications. A spin-filter kit consists of a screened cage surrounding an impeller shaft with very small filter pore openings that keep cells isolated outside the cage (Figure 93). Inside that rotating cage, a dip tube is provided for continuous withdrawal of culture broth. A media feed tube outside the cage provides a steady supply of fresh nutrients. Although pore openings vary from one manufacturer to the next, NBS spin filters come with 10-µm openings for suspension cultures and 75-µm openings for micro-carrier cultures.

Because of its gentle mixing nature, a spin filter is typically used with micro-carrier-dependent cell lines or those that are highly sensitive to shear. These mechanisms are ideal for use in production of secreted proteins because they keep harvested media cell-free, which simplifies purification in downstream processing. Over time, however, the screen material covering a spin-filter cage will become clogged with cell debris and require replacement. Culture run times are limited by this factor.

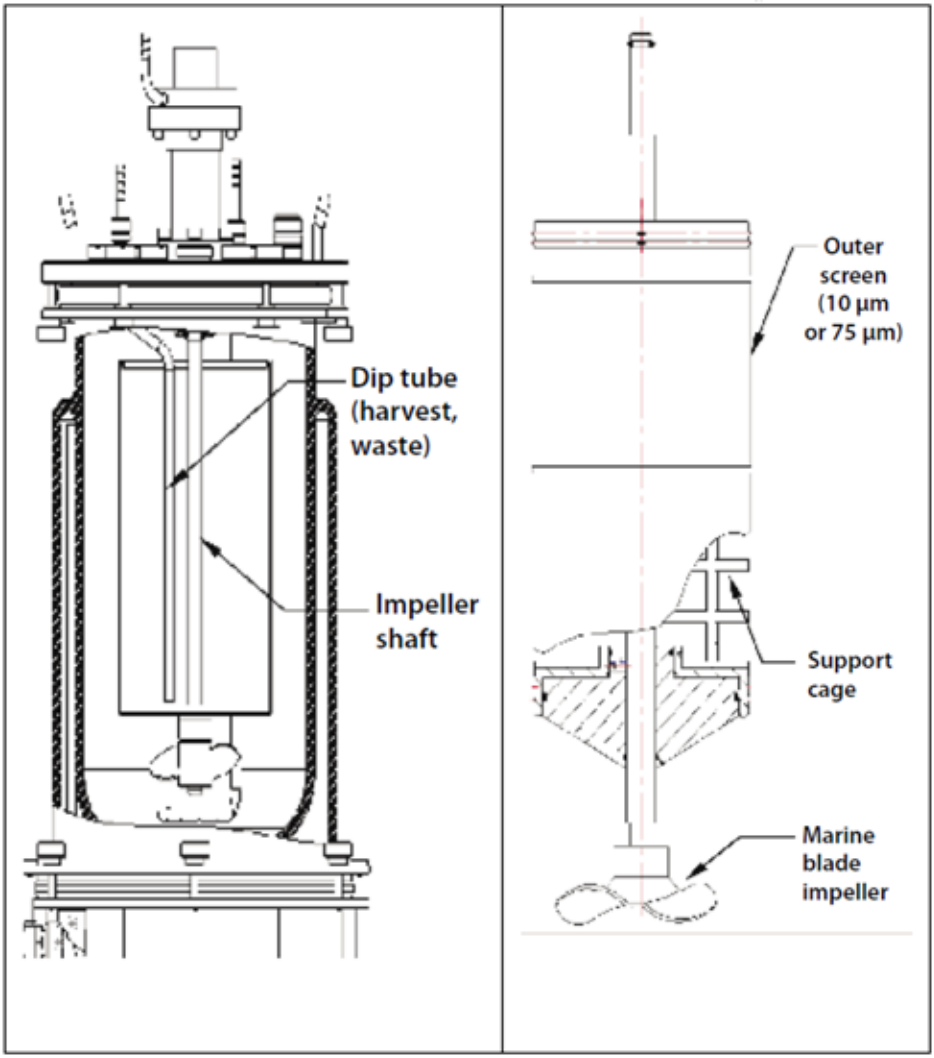

Fig. 93: Spin filter assembly

Special Impellers for Microcarrier Cultures

The CelliGen cell-lift impeller (Figure 94) designed and patented by New Brunswick Scientific provides uniform circulation for microcarrier cultures. This is an ultralow-shear impeller in which flow is caused by three discharge ports located on the impeller shaft. Rotation of those ports creates a low differential pressure at the base of the impeller tube, lifting microcarriers up

through the tube and expelling them out through its ports. This continuous recirculation loop keeps cells uniformly dispersed throughout a vessel. Gases are introduced through a ring sparger, which generates bubbles that pass along the impeller between the exterior of the inner tube and an outer membrane, known as the aeration cage. A mesh lining on the outer membrane of this cage has penetrations that are small enough (85 μm) to ensure that cells growing on the micro-carriers cannot pass through. Gas exchange occurs at the membrane–media interface, ensuring that cells remain in a bubble-free environment and are not subjected to shear due to bubble breakage. The bubbles are then expelled through two ports (located at the top of the impeller) into a second screened-in cage. A foam breaker directs air, supplied by a gas overlay, into the cage to break up foam. Cell-lift impellers are typically used in batch and fed-batch processes involving shear-sensitive animal cells. They can also be used for continuous perfusion processes when a decanting column(s) and media feed-in and broth pump-out setup are added.

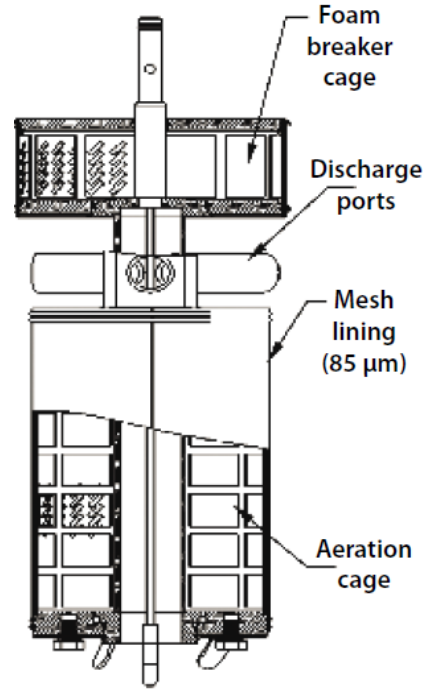

Fig. 94: Cell-lift impellers for microcarrier culture

Packed-Bed Basket Impellers

Another New Brunswick Scientific design is the packed-bed basket impeller used in the manufacture of secreted products from either anchorage-dependent or suspension cultures. A basket includes two horizontally positioned, perforated metal screens that extend to the walls of a bioreactor vessel (Figure 95). Enclosed between those screens, a bed of FibraCel disks serves as a solid support matrix for cell growth. Cells growing in the disk bed become immobilized on or between the disks, where they remain protected from external shear forces throughout each culture run. Media circulates by way of a hollow impeller tube with discharge ports positioned above the basket.

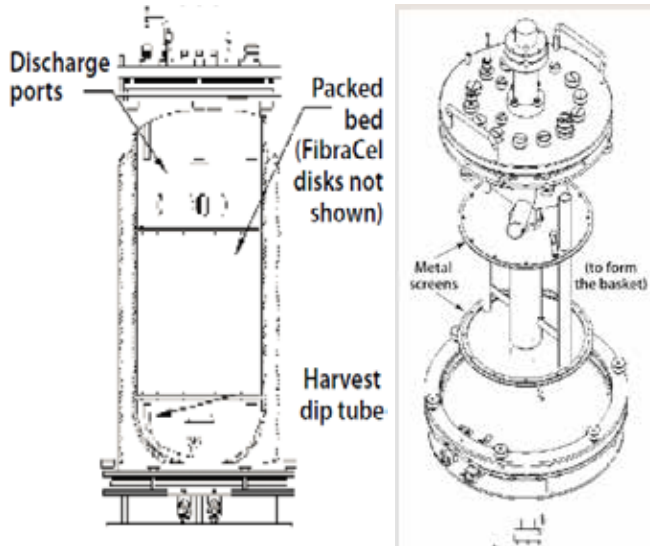

Fig. 95: Packed-bed basket impellers for secreted products

As with the cell-lift impeller, rotation of these discharge ports creates a low differential pressure at the base of the impeller tube, which circulates media throughout the system. The medium receives gases through a sparger located at the bottom of the inner tube, which protects cells from being exposed to the gas–liquid interface. This results in low turbulence and low shear stress for the culture. Exceptionally high cell densities are achievable with packed-bed baskets because of a high surface-to-volume ratio for cell growth provided by the disk bed coupled with an ability to use perfusion or medium-replacement techniques. Culture periods in excess of three months have been reported. By ensuring that cells remain entrapped in the bed, this system also simplifies protein harvesting from the resulting cell-free media.

Bubble Column Reactor

- Usually the height-to-diameter ratio is 4-6.
- Gas is sparged at the base through perforated pipes or plates or metal porous spargers.
- O_2 transfer, mixing and other performance factors are influenced mainly by gas flow rate and rheological properties of the fluid.
- Mixing and mass transfer can be improved by placing perforated plates or vertical baffles in the vessel.

Airlift Bioreactor

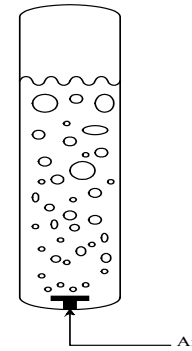

Fig. 96: A Bubble column Reactor

- Separated as two zones: the sparged zone is called the riser, and the zone that receives no gas is the downcomer. The bulk density in the riser region is lower than that in the downcomer region, causing the circulation (so circulation is enhanced if there is little or no gas in the downcomer).
- For optimal mass transfer, the riser to downcomer cross-sectional area ratio should be between 1.8 and 4.3.
- Highly energy efficient and productivities are comparable to those of stirred tank bioreactors.
- The rate of liquid circulation increases with the square root of the height of the airlift device. Consequently, the reactors are designed with high aspect ratios.

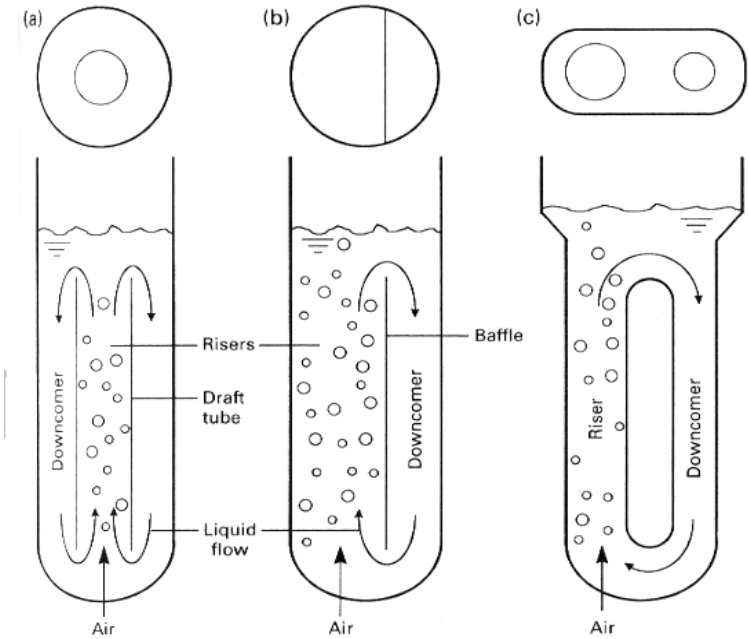

Fig. 97: Airlift bioreactor

- A gas-liquid separator in the head-zone can reduce the gas carry-over to the downcomer and hence increase the liquid circulation.

Fluidized Bed Reactor

- Suited for reactions involving a fluid-suspended particulate biocatalyst such as immobilized enzyme and cell particles.
- Similar to the bubble column reactor except that the top section is expanded to reduce the superficial velocity of the fluidizing liquid to a level below that needed to keep the solids in suspension. Consequently, the solids sediment in the expanded zone and drop back, hence the solids are retained in the reactor whereas the liquid flows out.

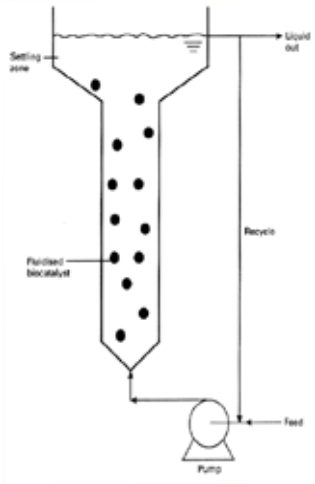

Fig. 98: Fluidized Bed Reactor

Packed Bed Bioreactor

- A bed of particles are confined in the reactor. The biocatalyst (or cell) is immobilized on the solids which may be rigid or macroporous particles.
- A fluid containing nutrients flows through the bed to provide the needs of the immobilized biocatalyst. Metabolites and products are released into the fluid and removed in the outflow.
- The flow can be upward or downward. If upward fluid is used, the velocity can not exceed the minimum fluidization velocity.

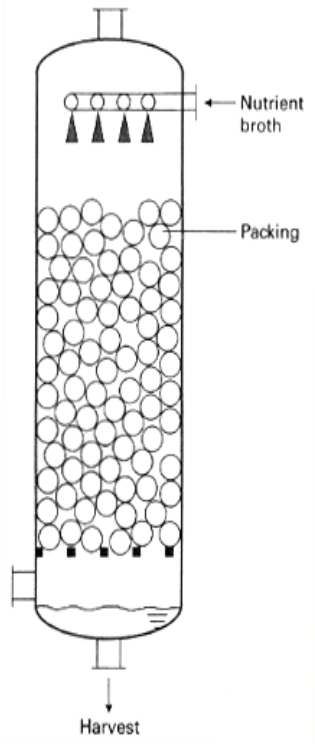

Fig. 99: The packed bed reactors

BIOREACTOR DESIGN FEATURES

- Medium or feed nozzle.
- Vertical sight glass and ports for pH, temperature and DO sensors.
- Connections for inoculum, acid and alkali (for pH control) and antifoam agents are located above the liquid level in the reactor vessel.

Fig. 100: BioFlo 6000® Sterilizable-In-Place Fermentor-Bioreactor (50 - 130 L)

Fig. 100A: BioFlo 110, 1.3 to 14 liters

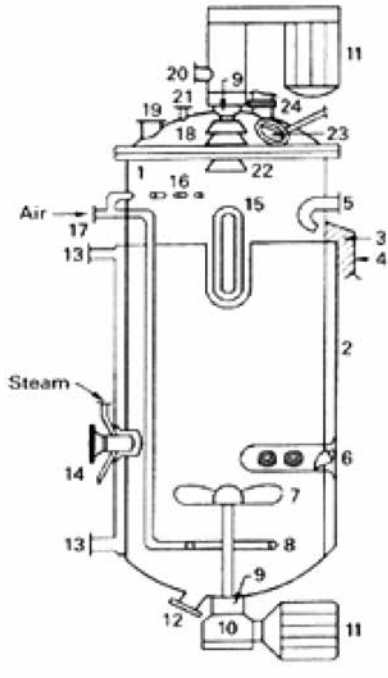

Fig. 101 A typical bioreactor: (1) reactor vessel; (2) jacket; (3) insulation; (4) shroud; (5) inoculum connection; (6) ports for pH, temperature and dissolved oxygen sensors; (7) agitator; (8) gas sparger; (9) mechanical seals; (10) reducing gearbox; (11) motor; (12) harvest nozzle; (13) jacket connections; (14) sample valve with steam connection; (15) sight glass; (16) connections for acid, alkali and antifoam chemicals; (17) air inlet; (18) removable top; (19) medium or feed nozzle; (20) air exhaust nozzle; (21) instrument ports (several); (22) foam breaker; (23) sight glass with light (not shown) and steam connection; (24) rupture disc nozzle.

- O_2 and other gases (CO_2 or NH_3 for pH control; N_2 for O_2 control) can be introduced through a sparger at the bottom.
- Foam breakers are used when antifoam is ineffective or the antifoam interferes with downstream processing (antifoam tends to foul the membrane during filtration).
- Can be sterilized in-place using saturated steam at a minimum absolute pressure of 212 k_Pa. Over–pressure protection is provided by a rupture disc on the top of the reactor, which cracks to relieve the pressure to avoid explosion.
- Maximum allowable working pressure is ≈377-412 k_Pa (absolute), allowable temperature is usually 150-180°C (>121°C for sterilization). The vessel should withstand full vacuum or it could collapse while cooling after sterilization.
- Usually made in Type 316L stainless steel, while the less expensive Type 304 (or 304L) is used for the jacket. The L grades contain less than 0.03% carbon, which reduces chromium carbide formation during welding and lowers the potential corrosion at the welds.
- The vessel should have as few internals as possible and should be free of stagnant areas where pockets of solids or liquids may accumulate.

DESIGN FOR STERILE OPERATION

Sterilization-in-place

- A bioreactor must be sterilized before inoculation because contamination is a common cause of process failure.
- For large bioreactors, *in situ* sterilization is common.
- The components should be able to be sterilized independently during fermentation if required.
- The aeration and exhaust groups must also be sterilized. The filters are rated for removing particles down to 0.22 μm or even 0.1 μm. Often the gas streams require two filter cartridges in series, with the first serving to protect the second filter.

Clean-in-place (CIP) Considerations

- Industrial bioreactors, including all pipings, should be cleaned in-

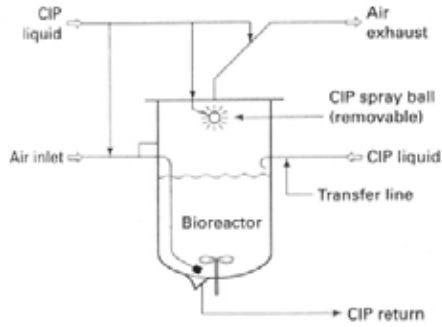

Fig. 103: Showing the CIP considerations

place using automated methods, so as to ensure consistency and reduce down-time.

- To remove solid particles and avoid sedimentation, a flow velocity of 2 m/s is preferred. Also, the piping should be free of dead space as much as possible. For thorough cleaning, the CIP solutions are sprayed through a spray ball. For cleaning with jet spray, pressures of 308 to 377 $k_p a$ (absolute) are optimal.
- Procedures:
  1. Pre-rinse for 5 min with deionized water (sufficient for bacteria, yeast and animal cell cultures).
  2. Circulate 1% (w/v) NaOH at 75-80°C through all product contact surfaces for 15-20 min. Discard the solution afterwards.
  3. Rinse at 25-35°C with deionized water to remove all alkali.
  4. Final wash with hot "water-for-injection" grade water.
- For stirred tank bioreactors, it is recommended to fill the vessel and agitate at Reynolds numbers of 10^8-$10^{8.5}$ during pre-rinse, alkali recirculation and the final rinse for 2-3 min (should be sufficient to dislodge adhering dirt or soil).

Note:

- Disposable bioreactors (*e.g.* Wave bioreactor, BelloCell) are gaining increasing interest due to smaller capital investment, easier operation and elimination of the CIP process.
- Water for Injection (WFI): high quality water subjected to the following treatment:

Note:

1. Ion exchanger need to be regenerated regularly by HCl and by NaOH
2. Reverse osmosis removes viruses, microorganisms, pyrogen and virtually all inorganic impurities.

MASS TRANSFER STEPS

- In bioreaction processes, substrates are consumed for the conversion. Typical substrates include carbon sources (*e.g.* sugar and oil), nitrogen sources (*e.g.* ammonia and amino acids) and electron acceptors (*e.g.* O_2).

Effects of Transfer Limitations

Two effects if one step is slower than the key kinetic reaction step:

1. The overall reaction rate is below the theoretical maximum, and the process output is slower than desired.
   - *Reversible effect*: for the production of gluconic acid from glucose by *Gluconobacter oxydans*, the O_2 transfer is limiting. Once O_2 limitation is relieved, there is no irreversible effect on this microorganism.
   - *Irreversible effect*: for the production of penicillin, O_2 limitation imposes an irreversible damage to the biosynthetic capacity of the cell.
2. The selectivity of the reaction is altered.
   - *Ex*: O_2 serves as an electron acceptor in the formation of baker's yeast from glucose. In the absence of O_2 the e^- will be directed to private resulting in the formation of ethanol and CO_2.
   - Transfer of oxygen involves a chain of mass transfer steps from a gas bubble. The slowest one is the rate-limiting step and determine whether the mass transfer rate would affect the overall process performance. When cells are well dispersed in the liquid and the bulk liquid is well mixed, step (iii) is the limiting step.

Transfer Across the Cell Envelope

- Transport across the cell envelope (may include cell wall and cytoplasmic membrane) can be limited.

- Three typical mechanisms:
  1. *Free diffusion*: passive transport down a concentration gradient
  2. *Facilitated diffusion*: as above but speeded up by a carrier protein
  3. *Active transport*: transport by a carrier protein with input of free energy

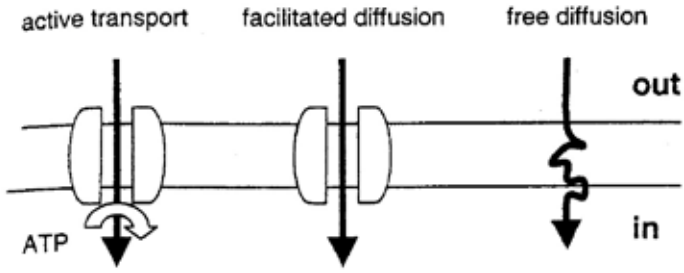

Fig: 104: The mechanisms of Mass transfer across the Cell Envelope

- The diameter of microbial cell itself is small (usually ≈1-5 μm) so diffusion inside the cell is more rapid and not a limiting factor.
- The transport barrier imposed by the membranes of intracellular organelles in eucaryotic cells usually does not limit the overall transport rate.

MASS TRANSFER EQUATIONS

Fundamentals—Fick's Equation

Developed by Adolf Eugen Fick (1829 - 1901), the Fick principle has been applied to the measurement of cardiac output. Its underlying principles may be also be applied in a variety of clinical situations.

The essence of the Fick principle is that blood flow to an organ can be calculated using a marker substance if the following information is known:

- Amount of marker substance taken up by the organ per unit time
- Concentration of marker substance in arterial blood supplying the organ
- Concentration of marker substance in venous blood leaving the organ

In Fick's original method, the "organ" was the entire human body and the marker substance was oxygen.

The principle may be applied in different ways. For example, if the blood flow to an organ is known, together with the arterial and venous concentrations of the marker substance, then the uptake of marker substance by the organ may then be calculated.

Variables

In Fick's original method, the following variables are measured:

- VO_2, oxygen consumption in ml of pure gaseous oxygen per minute. This may be measured using a spirometer within a closed rebreathing circuit incorporating a CO_2 absorber
- C_v, the oxygen concentration of blood taken from the pulmonary artery (representing deoxygenated blood)
- C_a, the oxygen concentration of blood from a cannula in a peripheral artery (representing oxygenated blood)

Equation

From these values, we know that:

$$VO_2 = (CO \times C_a) - (CO \times C_v)$$

where CO = Cardiac Output, C_a = Oxygen concentration of arterial blood and C_v = Oxygen concentration of mixed venous blood.

This allows us to say

$$CO = \frac{VO_2}{C_a - C_v}$$

and hence calculate cardiac output.

Note that $(C_a - C_v)$ is also known as the arteriovenous oxygen difference.

Assumed Fick Determination

In reality, this method is rarely used due to the difficulty of collecting and analysing the gas concentrations. However, by using an assumed value for oxygen consumption, cardiac output can be closely approximated without the cumbersome and time-consuming oxygen consumption measurement. This is sometimes called an assumed Fick determination.

A commonly-used value for O_2 consumption at rest is 125ml O_2 per minute per square meter of body surface area.

Underlying Principles

The Fick principle relies on the observation that the total uptake of (or release of) a substance by the peripheral tissues is equal to the product of the blood flow to the peripheral tissues and the arterial-venous concentration difference (gradient) of the substance. In the determination of cardiac output, the substance most commonly measured is the oxygen content of blood thus giving the arteriovenous oxygen difference, and the flow calculated is the flow across the pulmonary system. This gives a simple way to calculate the cardiac output:

$$\text{Cardiac Output} = \frac{\text{oxygen consumption}}{\text{arteriovenous oxygen difference}} \times 100$$

Assuming there is no intracardiac shunt, the pulmonary blood flow equals the systemic blood flow. Measurement of the arterial and venous oxygen content of blood involves the sampling of blood from the pulmonary artery (low oxygen content) and from the pulmonary vein (high oxygen content). In practice, sampling of peripheral arterial blood is a surrogate for pulmonary venous blood. Determination of the oxygen consumption of the peripheral tissues is more complex.

The calculation of the arterial and venous oxygen concentration of the blood is a straightforward process. Almost all oxygen in the blood is bound to hemoglobin molecules in the red blood cells. Measuring the content of hemoglobin in the blood and the percentage of saturation of hemoglobin (the oxygen saturation of the blood) is a simple process and is readily available to physicians. Using the fact that each gram of hemoglobin can carry 1.36 ml of O_2, the oxygen content of the blood (either arterial or venous) can be estimated by the following formula:

$$\text{Oxygen Content of blood} = [\text{Hb}]\ (\text{g/dl}) \times 1.36\,(\text{ml } O_2/\text{g of Hb}) \times O_2^{\text{saturation fraction}} + 0.0032 \times P_{O_2}(\text{torr})$$

Assuming a hemoglobin concentration of 15g/dl and an oxygen saturation of 99%, the oxygen concentration of arterial blood is approximately 200ml of O_2 per litre. The saturation of mixed venous blood is approximately 75% in health. Using this value in the above equation, the oxygen concentration of mixed venous blood is approximately 150ml of O_2 per litre.

Cardiac output may also be estimated with the Fick principle using production of carbon dioxide as a marker substance.

- Considering transport in solid phase, D is the effective diffusion coefficient which lumps the diffusion coefficient, the porosity of the solid, and the shape of the channels.
- For a flat plate with thickness d in a stationary fluid resistance against transport.
- For unsteady state:

$$D\frac{\partial^2 C}{\partial x^2} = \frac{\partial C}{\partial t}$$

- These equations consider the diffusion process only but not convection. In reality, convection is often encountered and flow pattern is not known, thus the mass transport often relies on empirical approach.

Mass Transfer between l-s Phase or l-g Phase (Two Film Theory)

For gas film transport:

$J_g = k_g(P - P_i)$ (mass transfer rate, not flux)

Liquid film transport:

$J_l = k_L a(C_i - C)$ (the main resistance to oxygen transfer)

k_L: liquid phase mass transfer coefficient (m/h)

$P_i \neq C_i$ but can be correlated by Henry coefficient H (bar · m^3/mol):

$P_i = HC_i$

From here, the volumetric mass transfer rate (J, mg/h/l), can be derived

$J = k_L a(C^* - C)$

- a (m^2/m^3): the gas-liquid interfacial area per unit liquid volume, or area per unit gross vessel volume in the bioreactor. When dealing with the transfer of O_2 from gas to liquid, *J* is called the OTR (oxygen transfer rate)
- $k_L a$ (1/h): volumetric transfer coefficient, because a and k_L are difficult to evaluate separately, $k_L a$ is often expressed together. Various

expressions of k_La can be found in literature. The value is typically $\approx 0.02 \sim 0.25\ s^{-1}$

- C*: saturated DO (in the case of oxygen transfer) concentration water under 1 atm air pressure is 0.2099 times that under 1 atm pure O_2.
- Oxygen uptake rate:

  $Q_O = q_O \cdot x$ (g/1·h) qo: specific oxygen uptake rate (g/g·s), x: cell concentration (g/l)
- q_o varies with cell species and nutritional environment

Table Solubility and Henry's constant for oxygen in pure water under 1 atm oxygen pressure

Temperature (°C)	*Oxygen solubility* (kg m^{-3})	*Henry's constant* (atm m^3 kg^{-1})
0	7.03×10^{-2}	14.2
10	5.49×10^{-2}	18.2
15	4.95×10^{-2}	20.2
20	4.50×10^{-2}	22.2
25	4.14×10^{-2}	24.2
26	4.07×10^{-2}	24.6
27	4.01×10^{-2}	24.9
28	3.95×10^{-2}	25.3
29	3.89×10^{-2}	25.7
30	3.84×10^{-2}	26.1
35	3.58×10^{-2}	27.9
40	3.37×10^{-2}	29.7

Table Solubility of oxygen in water under 1 atm air pressure

Temperature (°C)	*Oxygen solubility* (kg m^{-3})
0	1.48×10^{-2}
10	1.15×10^{-2}
15	1.04×10^{-2}
20	9.45×10^{-3}
25	8.69×10^{-3}
26	8.55×10^{-3}
27	8.42×10^{-3}
28	8.29×10^{-3}
29	8.17×10^{-3}
30	8.05×10^{-3}
35	7.52×10^{-3}
40	7.07×10^{-3}

- For C> C_{crit} (critical oxygen concentration), q_o is a constant maximum
- For C< C_{crit}, q_o (usually 5-10% air saturation) is approximately linearly dependent on C.

At Steady State,

$$k_L a(C^* - C) = q_o \cdot x$$

This equation can be used to predict the response of fermenter to changes in mass transfer operating conditions, for example, if $k_L a$ is raised by increasing stirrer speed→C must rise.

Measurement of $k_L a$

1. Oxygen-balance method: (based on steady state measurement)

- at s.s.

$$J = \frac{1}{V_L}(F_g C)_i - (F_g C)_o)$$

where l.h.s: rate of oxygen transfer from gas to liquid

r.h.s.: difference in oxygen flow between inlet (subscript i) and outlet (subscript o). V_L, volume of liquid; F_g, volumetric gas flow rate; C, gas phase concentration of O_2

- Because gas phase concentrations are usually measured as partial pressures, ideal gas law can be incorporated:

$$J = \frac{1}{RV_L}((\frac{F_g p}{T})_i - (\frac{F_g p}{T})_o)$$

where p is the partial pressure at the inlet and outlet. The difference between p_i and p_o is usually small thus pi and p_o need to be measured very accurately (*e.g.* by mass spectrometry).

- For known p, F_g, V_L, R and T, J is obtained, and $k_L a$ can be calculated from $J = k_L a(C^* - C)$ when C* and C are measured.

2. Dynamic method: (based on unsteady state measurement)

- At time t_0, the broth is de-oxygenated by sparging N_2 or by stopping the air flow if the culture is consuming O_2 → C drops
- Air is then pumped into the broth at a constant flow rate → C increases and reaches a s.s. value, $\bar{C}$. C_1 and C_2 are two oxygen concentrations measured at t_1 and t_2.

- During the re-oxygenation step (unsteady state)

$$\frac{dC_{AL}}{dt} = k_L a\left(C^*_{AL} - C_{AL}\right) - q_O x$$

- At s.s., $k_L a(C^* - \bar{C}) = q_o \cdot x$ is substituted into the above equation to yield

Assume $k_L a$ is a constant, integrating the above equation from t_1 to t_2 yields

$$k_L a = \frac{\ln\left(\frac{\bar{C} - C_1}{\bar{C} - C_2}\right)}{t_2 - t_1}$$

Gas-Liquid Mass Transfer in Real Systems (O_2 transfer)

- Bubble size is critical in determining oxygen transfer, smaller bubbles leads to:

$$e = \frac{V_G}{V_L + V_G}$$

V_G: volume of gas bubbles in the reactor, V_L: volume of liquid

- Two extreme cases:
  1. Coalescing liquid (a liquid which greatly stimulates bubble coalescence): mass transfer is poorest
  2. Non-coalescence fluid: highest mass transfer.

Stirred tank reactor:

- Sparged gas is usually rapidly collected in the gas cavities behind the rotating impeller blades. The cavities flow in a highly turbulent vortex and the gas is dispersed into smaller bubbles. These follow the liquid flow, but will also rise to the surface. They will coalesce in areas that are relatively calm and re-disperse in places where the shear stress is high. A part of the bubbles is re-circulated into the cavities and the rest escapes at the surface.
- Empirical values for gas hold-up

$\varepsilon = 0.13 \cdot (P/V)^{0.33} (v_g p_0 / p)^{0.67}$ (for coalescing fluid)